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A Biomimetic Approach to Lanthionines

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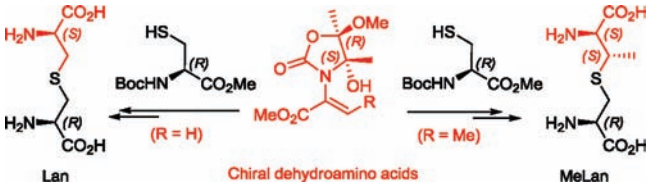
ABSTRACT



The asymmetric sulfa-Michael additions of appropriately protected L- and D-cysteine derivatives to new chiral dehydroamino acid derivatives have been developed as key steps in the synthesis of biologically important cysteine derivatives, such as lanthionine (Lan) and β-methyllanthionine (MeLan), which are unusual bis-α-amino acids found in the emerging lantibiotics such as nisin.

One of the most important features of the natural amino acid cysteine (Cys) is related to the nucleophilicity of its SH moiety. For example, this thiol group can stabilize the tertiary structure of significant bioactive peptides, due to its participation in disulfide bonds, as well as play an important role as a nucleophile in Michael-type reactions onto dehydroamino acids to generate peptide sequences.[1]

In this sense, significant lantibiotics as nisin, duramycin, and subtilin[2] contain in their structures lanthionine or methyllanthionines, which are unusual bis-α-amino acids that consist of two alanyl residues bridged by a thioether linkage. The biosynthetic origin of these structural features involves the initial dehydration of specific Ser and Thr residues in the prelantibiotic peptide and the subsequent stereoselective enzyme-mediated Michael addition of cysteine thiols to the newly formed dehydroalanine (Dha) and dehydrobutyrine (Dhb) residues, respectively[3] (Figure S1, Supporting Information (SI)). In contrast to the labile disulfide bond of cystine, the monosulfur bridge of lanthionine derivatives is chemically far stronger. For this reason, thioether-bridged peptides[4,5] have therefore been incorporated into medicinally relevant peptides.

On the other hand, taking into account that the increasing problem of antibiotic resistance has highlighted the imperative demand for novel antimicrobial agents, peptides containing the thioether bridge, such as lantibiotics, have emerged as important tools to address this problem.[6] A convenient strategy toward the synthesis of lantibiotics

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involves the incorporation of orthogonally protected lanthionines as building blocks in their solid-phase synthesis.[7] Therefore, several synthetic approaches to lanthionine (Lan) and β-methyllanthionine (MeLan) monomers have been described in recent years.[2,8] Moreover, the synthesis of isomers namely norlanthionines (*nor*-Lan)[9] and α-methylnorlanthionines (α-Me-*nor*-Lan),[10] which consist of an alanyl and a β-alanyl or a α-methyl-β-alanyl residues, respectively, have been reported and *nor*-Lan has been incorporated in a cyclic peptide analog of the ring C of lantibiotic nisin.[11]

Taking into account the importance of these bis-α-amino acids, the development of a new and efficient synthesis of these systems seems to be of interest. Herein, we report a biomimetic approach to Lan and MeLan exploiting the high nucleophilicity of the sulfhydryl group of Cys in a stereoselective Michael reaction of the convenient protected cysteine derivative onto chiral dehydroalanine and dehydrobutyrine methyl esters (Figure S1, SI).

Despite being one of the most important and widely used synthetic tools in organic synthesis,[12] there are relatively few reports on the use of the Michael addition of nucleophiles to chiral α,β-dehydroamino acids,[13] and in most cases chirality is present in both amino and carboxylic acid groups, involving cyclic systems such as oxazolidinones or dehydrodiketopiperazines.[14]

In particular, several approaches have been documented for diastereo- and enantiocontrol in the conjugate addition of sulfur-based nucleophiles to Michael acceptors,[15] and to the best of our knowledge, there are only a few examples of sulfa-Michael additions onto chiral dehydroamino acids.[16] The use of Michael additions of cysteine onto Dha/Dhb in linear lantibiotic precursors (in the absence of enzymes) is well documented[16d−g] and these reactions frequently are stereoselective when a "natural" linear sequence is used. Likely, the closest work to the present study is the convergent synthesis of peptide conjugates using the dehydroalanine moiety of several dehydropeptides for chemoselective ligations; however no asymmetric induction by the chiral backbone of the peptide was observed in the protonation of the enolate intermediate formed by the initial Michael addition.[17]

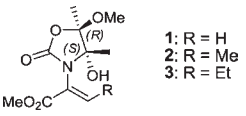

Figure 1. Dehydroamino acids **1**, **2**, and **3**.

We recently reported an efficient synthesis of chiral dehydroamino acid derivatives **1**−**3** as potential chiral building blocks for the Michael addition[18] (Figure 1). Now, in this communication, their absolute configurations are confirmed by X-ray analysis (Figure S2, SI). It is important to highlight that, while several chiral dehydro amino acids have been used for developing stereoselective Michael reactions,[16,19] there are few examples concerning chiral building blocks in which the dehydroamino acid displays chirality exclusively in the amino moiety, as a chiral enecarbamate.[20]

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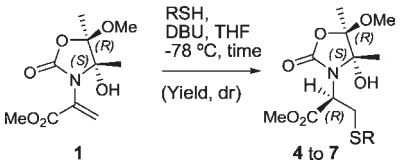
To demonstrate the potential synthetic use of these systems as new chiral building blocks, we performed the Michael reaction of dehydroalanine **1** with commercial *S*-nucleophiles, such as propane-1-thiol, cyclohexanethiol, *tert*-butylthiol, and thiophenol[21] (Table 1).

Table 1. Michael Reactions between Chiral Dehydroalanine **1** and Different *S*-Nucleophiles



entry	R	compd	Time (min)	Yield (%)[a]	dr
1	Pr	**4**	15	95	>95:5
2	Cy	**5**	60	86	>95:5
3	^{t}Bu	**6**	180	82	>95:5
4	Ph	**7**	60	73	>95:5

[a] Yield of products after column chromatography.

We started our experiments using THF as a solvent in the presence of DBU as a base to generate in situ the corresponding thiolates. Initially and using these conditions, we assayed the Michael reaction of dehydroalanine **1** with propane-1-thiol at 0 °C, giving a mixture of two isomers. Therefore, we carried out the reaction at −78 °C, giving, after 15 min, exclusively one product corresponding to structure **4**. The diastereomeric ratio (dr) was assumed to be >95:5, because in the ^{1}H NMR spectrum corresponding to the crude of the reaction only one diastereoisomer was observed. After column chromatography, **4** was obtained in a 95% yield (entry 1, Table 1).

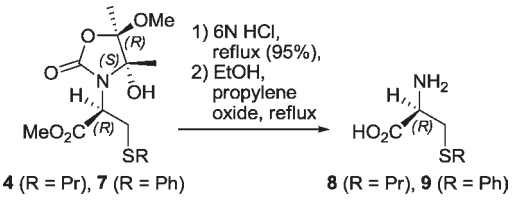
Similar features were observed when cyclohexanethiol, *tert*-butanethiol, and thiophenol were used as *S*-nucleophiles giving compounds **5**, **6**, and **7**, respectively (entries 2, 3, and 4, Table 1). The sole difference was the slight decrease in yield of the reactions, probably due to the steric volume of these nucleophiles.

The absolute configuration of the Michael adduct **6** was determined unambiguously by X-ray analysis, showing the new sterocenter created in the Michael reaction an (*R*)-configuration (Figure S3, SI).

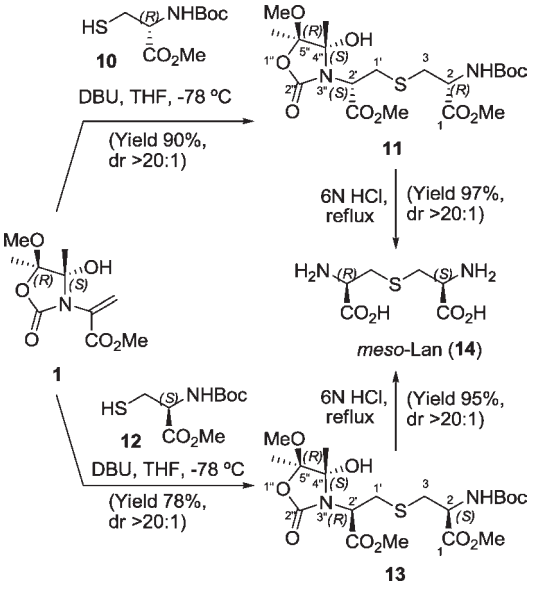
In the cases of **4** and **7**, the absolute configurations of the new stereocenters were determined by transformation of these compounds into the well-known amino acids *S*-propylcysteine **8** and *S*-phenylcysteine **9**, respectively, and comparing the optical rotations measured with those appeared in the literature.[22] These transformations were carried out using acid hydrolysis in an aqueous 6 N HCl solution at reflux, followed by treatment with propylene oxide in ethanol (Scheme 1).

In view of the excellent results obtained in these preliminary reactions, we assayed the Michael reaction with

Scheme 1. Synthesis of *S*-Propyl- and *S*-Phenylcysteines

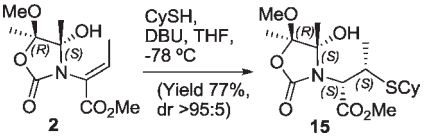


Scheme 2. Synthesis of *meso*-Lanthionine



L- and D-cysteine derivatives as *S*-nucleophiles and dehydroalanine **1** as the Michael acceptor.[23] Therefore, *N*-Boc-L-cysteine methyl ester **10** was reacted with chiral Michael acceptor **1** using DBU as a base and THF as a solvent at −78 °C for 30 min, giving exclusively compound **11**, a protected Lan, in an excellent yield (90%) with a dr >95:5, as we determined by ^{1}H NMR spectroscopy (Scheme 2).

Scheme 3. Michael Reaction between Chiral β-Substituted Dehydrobutyrine **2** and Cyclohexanethiol



Nevertheless, in the case of *N*-Boc-D-cysteine methyl ester **12**, because it is not commercially available, it had to

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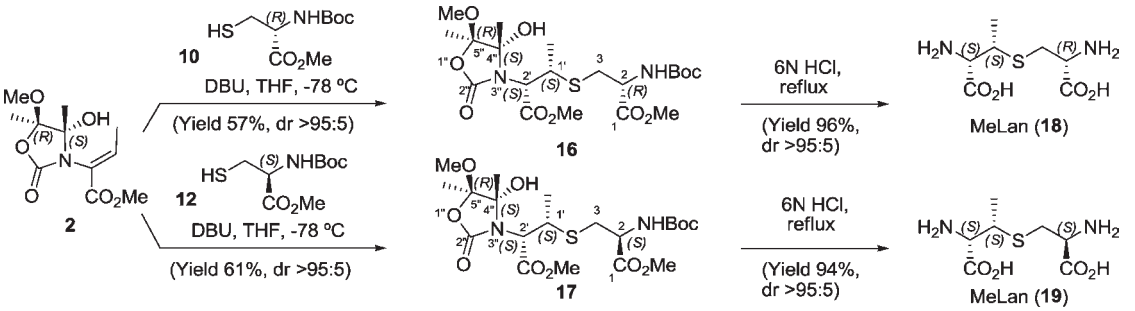
Scheme 4. Stereodivergent Syntheses of MeLan **18** and **19** Using a Chiral Sulfa-Michael Addition as a Key Step



be synthesized following the published procedure,[24] starting from D-cystine, whose amino and carboxylic acid groups were protected as Boc and methyl ester, respectively. Cleavage of the disulfide bond with tri-*n*-butylphosphine (Bu_3P) gave the corresponding D-cysteine derivative **12**, which was used as an *S*-nucleophile with dehydroalanine **1** in the Michael reaction, giving exclusively one product corresponding to the compound **13** (Scheme 2). Both chiral precursors of Lan **11** and **13** were subjected to acid hydrolysis giving, surprisingly, the same compound, the bioactive *meso*-lanthionine (*meso*-Lan) (Scheme 2). With these hydrolysis experiments we could assert the absolute configurations of the new stereocenters created in both double asymmetric Michael additions.[8e] Thus, a chiral *S*-nucleophile of (*R*)-configuration induces an (*S*)-configuration in the new stereocenter, while the use of a chiral *S*-nucleophile of (*S*)-configuration induces an (*R*)-configuration in the new stereocenter. Thus, this procedure allowed us the easy synthesis of bioactive *meso*-Lan using, as a key step, the double asymmetric *S*-Michael reaction between a chiral dehydroalanine and L- or D-cysteine derivatives.

In a further step and to check the effect of the β-substitution in the chiral Michael acceptor, we planned to expand this reactivity to the β-substituted dehydroamino acid **2**. Therefore, we carried out the Michael addition of dehydrobutyrine **2** and cyclohexanethiol as an *S*-nucleophile using THF as a solvent and DBU as a base. After the reaction was stirred for 4 h, only one diastereoisomer (compound **15**) was obtained with a 77% yield (Scheme 3).

The absolute configuration of **15** could be determined unambiguously by X-ray analysis, showing the (*S,S*)-configuration of two new sterocenters created in the Michael reaction (Figure S4, SI).

With this excellent result in hand, we decided to study the double asymmetric Michael additions between this chiral dehydrobutyrine **2** and the protected cysteines **10** and **12** as a source of β-methyllanthionines (MeLan), carrying out the reactions under the same conditions used above (Scheme 4).

Although the yield of the reactions slightly decreased to 57% and 61% for the products **16** and **17**, respectively, the diastereoselectivities achieved were excellent, since in each case only one product was obtained out of the four possibilities. In both cases the induction of diastereoselectivity was the same, producing (*S,S*)-configurations in the two new stereocenters created. These Michael adducts were easily transformed, by acid hydrolysis, into the corresponding MeLan **18** and **19** (Scheme 4). This fact allowed us to confirm the absolute configurations of the stereocenters created in the Michael additions,[8f] which corresponded to (*S,S*).

Taking into account the different behaviors of *S*-nucleophiles in these Michael reactions and the inherent difficulty in predicting the stereochemical outcome, in future works we will undertake an extensive mechanistic and theoretical study to elucidate first the role of the β-substitution in the chiral dehydroamino acid building block and also the role of chirality in the *S*-nucleophile.

In summary, we have carried out the stereoselective synthesis of bioactive *meso*-Lan (**14**) and optically active MeLans (**18** and **19**), all as hydrochloride derivatives, using a straightforward strategy based on the double asymmetric sulfa-Michael addition of L- and D-cysteine derivatives onto chiral dehydroamino acid derivatives **1** and **2**, which could be considered as a biomimetic approach.

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Supporting Information Available. Experimental details, as well as spectroscopic characterization of all new compounds and X-ray data for compounds **1**, **3**, **6**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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